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(54) Use of felbamate for the manufacture of a medicament for the treatment of neuropsychopharmacological disorders.

(57) The use of 2-phenyl-1,3-propanediol dicarbanate for the manufacture of a medicament for treating and controlling the symptoms of, or for the prevention and control of neurodegenerative disorders associated with or resulting from excessive activation of the N-methyl-D-aspartate receptor complex in human or other warm blooded animal patients.

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The present invention relates to pharmaceutical compositions containing 2-phenyl-1,3-propanediol dicarbamate (felbamate) as an active component and to methods for the prevention and control of the symptoms of neuropsychopharmacological disorders through the use of such compositions. More particularly, the present invention relates to methods for treating and controlling the symptoms of neuropsychopharmacological disorders and neurodegenerative diseases associated with or resulting from excessive activation of the N-methyl-D-aspartate receptor complex.

Neurochemical and electrophysiological studies have demonstrated that glycine modulates excitatory neurotransmission in the central nervous system. These actions are mediated through strychnine-insensitive glycine receptors with markedly different structural requirements for ligand binding and regional distribution than strychnine-sensitive sites associated with the role of glycine as an inhibitory neurotransmitter.

Neurotransmissions mediated through the N-methyl-D-aspartate (NMDA) receptor supramolecular complex are believed to be associated with numerous pathologic and physiologic mechanisms which include: kindling development, ischemic neuronal injury, synaptogenesis, spatial learning and long-term potentiation. Regulation of these neuronal mechanisms by NMDA-mediated processes is believed to involve activation of a receptor-gated ion channel.

Evidence now available clearly indicates that the NMDA complex is regulated, at least in part, by the amino acid glycine. Glycine has been shown to increase NMDA evoked currents in various tissues by increasing the opening frequency of the NMDA channel. Thus, NMDA-induced influx and intracellular accumulation of calcium are stimulated by glycine, which interacts with its own distinct site on the receptor. It is believed that the accumulation of intracellular calcium is implicated in the various neuropathologies.

Up to the present time, all drugs used in the treatment of neuropsychopharmacological disorders and neurodegenerative diseases function as prophylactics against the symptoms of these disorders and diseases as opposed to being curatives.

In accordance with the present invention, it has been established that felbamate interacts with strychnine-insensitive glycine receptors and that neuroprotective effects of felbamate are at least in part mediated through a strychnine-insensitive glycine receptor mechanism.

Felbamate is a well-known pharmaceutical compound having been described together with methods for its manufacture and use in US-A- 2,884,444; US-A-4,868,327; US-A- 4,978,680; US-A- 5,055489; US-A-5,072,056 and US-A- 5,082,861.

It has now been found possible to provide methods and compositions for the treatment and control of the symptoms of neuropsychopharmacological disorders and neurodegenerative diseases.

It has also been found possible to provide methods and compositions for the treatment and control of the symptoms of neuropsychopharmacological disorders and neurodegenerative diseases resulting from excessive activation of the NMDA through the use of felbamate.

Accordingly, in the present invention it has been found that felbamate chemically described as 2-phenyl-1,3-propanediol dicarbamate is a compound which has demonstrated superior properties with respect to the treatment of disorders such as, for example, hypoxia, either alone, e.g. CO poisoning, near drowning; or combined with ischemic blood flow reduction, e.g. cardiac arrest, stroke; anxiety and neurodegenerative diseases, e.g. Guam ALS, Parkinson's disease, Alzheimer's disease, dementia and lathyrism.

According to the present invention there is provided the use of 2-phenyl-1,3-propane diol for the manufacture of a medicament for treating and controlling the symptoms of, or for the prevention and control of neurodegenerative disorders associated with or resulting from excessive activation of the N-methyl-D-aspartate receptor complex in human or other warm blooded animal patients.

The compositions of the present invention may take any of a variety of forms although they are intended primarily for oral use and are suitable for forming into pills, capsules and tablets by well-known practices. Such forms may be dosage unit forms. When the active ingredient is in the form of a solid, a typical tablet composition comprises 500 milligrams of 2-phenyl-1,3-propanediol dicarbamate intermixed in a dry pulverulent state with suitable solid carriers and diluents.

Solid carriers and diluents suitable for use include sugars such as, for example, lactose and sucrose; cellulose derivatives such as, for example, carboxymethyl cellulose, ethyl cellulose, methyl cellulose; gelatin including hard and soft gelatin capsules, talc, cornstarch, stearic acid and magnesium stearate.

The percentage of 2-phenyl-1,3-propanediol dicarbamate in the compositions may be varied over wide limits and the quantity of medicament furnished by each individual tablet or capsule is relatively unimportant since the indicated total daily dose can be reached by administering either one or a plurality of capsules or tablets.

In general, an effective daily dose of the active ingredient is in the range of from about 100 milligrams to about 5 grams.

Felbamate (2-phenyl-1,3-propanediol dicarbamate) has a very favourable preclinical profile characterized by a substantial margin of safety (protective index 16.9 - 19.1).

The present invention will now be further described with reference to, but is in no manner limited to, the following Examples.

In the Examples, the ability of felbamate to interact with strychnine-insensitve glycine receptors is demonstrated. More particularly, in Example I, the radio ligand binding to glycine receptors is demonstrated using the antagonist [^{3}H]5,7-dichlorokynurenic acid, commonly referred to as [^{3}H]5,7DCKA which has been reported to bind with high affinity to glycine receptors in rat membranes. This radiolabelled compound is particularly suitable for purposes of demonstrating the present invention because of its high affinity and reliability within and between experiments.

In Example II, the neuroprotective effects of felbamate against hypoxic damage are demonstrated in the hippocampal slice model. This in vitro model simulates in vivo hypoxic-ischemic neuronal insults such as occur in stroke and other cerebral ischemic events.

EXAMPLE I

Male Sprague-Dawley rats weighing 150-200 g are housed under a 12-hour light/dark cycle with access to food and water ad libitum. Animals are anesthetized with CO_2 and sacrificed by decapitation. Brains are immediately removed and forebrains dissected, weighed and placed in 10 volumes (original wet weight volume) of 5 mM Hepes/4.5 mM Tris buffer (HTS; pH 7.8) containing 0.32 M sucrose. Tissue preparation is performed at about 4°C unless otherwise stated. Tissues are homogenized using a Brinkmann Polytron (setting 6, 30 seconds), diluted to 50 volumes in HTS-sucrose, and centrifuged at 1,000 xg for 10 minutes. The resultant pellet (P1) is discarded and supernatant centrifuged at 20,000 xg for 20 minutes. The resultant pellet (P2) is re-suspended in HTS and centrifuged at 8,000 xg for 20 minutes. The supernatant and outer buffy coat (remaining pellet core discarded) is centrifuged at 20,000 xg for 20 minutes. The resultant pellet (P2/P3) is resuspended in HTS containing 1 mM EDTA and centrifuged at 20,000 xg for 20 minutes. The P2/P3 pellet is resuspended in HTS and the "washing" procedure is repeated two more times. The P2/P3 pellet is then re-suspended in 5 volumes of HTS, frozen on dry-ice and stored at -80°C for at least 72 hours prior to binding assay.

At the time of the assay, the appropriate amount of tissue is thawed and re-suspended in 50 mM Hepes-KOH buffer (pH 8.0 at 4°C) and "washed" twice by re-suspension and centrifugation at 20,000 xg for 20 minutes. The binding of [^{3}H]5,7DCKA is performed as described by Baron et al. Env. J. Pharmacol 206: 149-154 (1991). Assays are performed at 4°C using quadruplicate samples in a total volume/tube of 1-ml consisting of: 500 µl membrane suspension (200-300 µg protein/assay tube) in 50 mM Hepes-KOH buffer (pH 8.0 at 4°C), 100 µl [^{3}H]5,7DCKA solution (19-21 nM; specific activity 18.2 Ci/mmol), 100 µl drug or buffer, and 300 µl buffer. Glycine or 7-chlorokynurenic acid (100 uM) is used to define .pl70 non-specific binding. Felbamate, solutions of 10mM and 100 mM, are dissolved in 40-50% and 100% DMSO, respectively, and serial dilutions are performed. Binding reactions are initiated by adding the tissue homogenate and terminated after a 15-minute incubation period by rapid filtration through Brandel GF/B filters using a Brandel M-24 cell harvester. This filtration is followed by two 5-ml rinses with ice-cold buffer. Radioactivity is monitored in a Beckman LS 5801 liquid scintillation counter.

Inhibition of [^{3}H]5,7DCKA binding by glycine (10 nM-10µM) or felbamate (10 µM-10mM) is performed to assess the potencies of these compounds for strychnine-insensitive glycine receptors. Displacement curves and IC_{50} values were analyzed using Graphpad (ISI Software, Philadelphia, PA). The K_1 values were calculated using the equation: $K_1=IC_{50}/1+[L]/K_D$, where [L] is the concentration of [^{3}H]5,7DCKA (19-21 nM) used in the assay and K_D is the dissociation constant of [^{3}H]5,7DCKA (69 nM).

Displacement of [^{3}H]5,7DCKA by glycine (10 nM 10 µM) yielded an IC_{50} of 371 nM (Figure 1). Inhibition of [^{3}H]5,7DCKA by felbamate (10 µM-10mM) resulted in an IC_{50} value of 374 µM (Figure 2). The IC_{50} shown in Figure 2 is generated by combining and then plotting on a single graph the felbamate displacement data from three separate experiments. A Hill slope (-0.93) of these data demonstrated unity and indicated interaction with a single population of sites. The IC_{50} value (mean +/- SEM) from the felbamate displacement experiments presented is calculated to be 477 +/- 49 µM. The K_1 estimates for inhibition of [^{3}H-]5,7DCKA were: glycine, 284 nM (Figure 1); felbamate, 289 µM; and felbamate (mean +/- SEM), 368 +/- 37 µM (Figure 2).

The IC_{50} value obtained from glycine inhibition of [^{3}H]5,7DCKA (Figure 1) corresponds well with previously reported data. Moreover, results obtained demonstrate tha felbamate interacts with strychnine-intensitive glycine receptors at relatively high concentrations. These conclusions are based on displacement data illustrating that felbamate competitively inhibits [^{3}H]5,7DCKA) binding to rat forebrain membranes (Figure 2).

EXAMPLE II

Sprague-Dawley rats are briefly anesthetized with Halothane and then decapitated. The brain is removed and the hippocampus dissected. Transverse hippocampal brain slices of 475 microns are sectioned with a McIlwain tissue chopper. Slices are then incubated in a temperature controlled chamber of 34°C while being perfused with an artificial cerebral spinal fluid (NaCl 126 mM; KCl, 4; KH_2PO_4, 1.4;

$MgSO_4$, 1.3; $CaCl_2$, 2.4; $NaHCO_3$, 26 and glucose, 4) saturated with 95% O_2 and 5% CO_2.

After an initial one hour equilibration period, the slices are tested for electrophysiological function. Electrical stimulation is given in the region of the CA3 collaterals with a bipolar twisted wire electrode. Evoked responses are recorded extracellularly in the pyramidal cell layer of the CA1 region. Stimulation is given for a duration of 40 microseconds in square wave pulses. The peak-to-peak amplitude of the resultant evoked potential response is then monitored.

Hippocampal slices from one animal are placed in two chambers. One chamber is used as a control and receives standard artificial cerebral spinal fluid (ACSF) while the second receives felbamate. In each chamber, one slice is stimulated every 30 seconds to monitor evoked potential response. Other slices in the chamber are not continuously stimulated. In these latter slices, designated as non-stimulated, the evoked potential response is assessed only at the beginning and end of the experiment. Only slices with initial evoked potentials of 3 mV or greater amplitude are included for testing.

The experimental chamber is perfused with oxygenated artifical cerebral spinal fluid (ACSF) containing felbamate for 30 minutes before the initiation of hypoxic conditions. The control chamber continues to receive oxygenated ACSF without felbamate during this period. Hypoxic conditions are then initiated simultaneously in both chambers by changing to perfusing media saturated with 95% N_2 and 5% CO_2. The experimental chamber receives nitrogenated ACSF with felbamate while the control chamber receives nitrogenated ACSF without felbamate.

The duration of hypoxic exposure for both chambers is determined by the disappearance of the hypoxic injury potential (HIP) in the control stimulated slice. This potential appears during hypoxia after the disappearance of the original evoked potential. Although hippocampal slices can vary in their temporal response to hypoxia, the HIP is a reliable marker of permanent hypoxic injury. For this reason, the disappearance of the HIP is chosen to determine length of hypoxic exposure. Hypoxia is continued in both chambers for 5 minutes beyond the disappearance of the HIP in the stimulated control slice.

After hypoxic exposure, slices are monitored through one hour of recovery with oxygenated ACSF. The felbamate chamber receives oxygenated ACSF with felbamate for the first 15 minutes of this recovery and then standard oxygenated ACSF for the remaining 45 minutes of recovery. After a one-hour recovery period, the percentage of evoked potential amplitude recovery is assessed in both stimulated and non-stimulated slices. This percentage is calculated as the evoked potential amplitude after recovery divided by the evoked potential amplitude prior to hypoxic exposure.

The results of the foregoing procedure are as follows:

a. Pre-hypoxic Incubation.

Felbamate perfusion produced occasional transient collapse and disappearance of the evoked potential, but no evidence of toxicity as evidenced by permanent potential loss was seen.

b. Hypoxic Neuroprotection.

Significant neuroprotection against hypoxia is seen at felbamate concentrations of 380 μM, 840 μM, 1,300 μM and 1,700 μM.

This protective action is assessed by several measures. First, evoked potential recovery is assessed in both stimulated and non-stimulated slices. Additionally, hypoxic protection is calculated for both stimulated and non-stimulated slices. This measure is calculated as the damage seen in control slices minus the damage seen in experimental (felbamate) slices divided by the damage seen in control slices. For these purposes damage is defined as 100 percent minus percent recovery. The determination of hypoxic protection helps take into account any survival seen in control slices. Lastly, counts of total surviving slices were made. For this purpose a minimal amplitude criteria of 3 mV was used as the indicator of a surviving slice.

In stimulated control slices, hypoxic exposure resulted in near complete loss (1.0% mean recovery) of the population spike, while slices treated with 4, 190, 380, 840, 1,300 and 1,700 μM felbamate showed respectively 2%, 6%, 13%, 46%, 95% and 96% recovery. This recovery is significant at p 0.05 for 840 μM and significant at p 0.001 for concentrations of 1,300 and 1,700 μM. Since recovery in the stimulated control slices is minimal, calculated protection was essentially identical to recovery. Counts of surviving stimulated slices indicated substantial felbamate protection at doses of 1,300 and 1,700 μM. Interestingly, 1,700 μM felbamate delayed the appearance of the HIP by 14.5 minutes (p 0.05) but did not affect the disappearance of the evoked potential.

In non-stimulated control slices, recovery from hypoxic exposure shows greater recovery than stimulated control slices and a mean non-stimulated control recovery of 30% is seen. Calculated protection with felbamate concentrations of 4, 190, 380, 840, 1,300 and 1,700 μM, show respective values of 4%, 63%, 48%, 100% and 100%. Additionally, counts of surviving non-stimulated slices showed substantial protection.

The foregoing Example indicates a significant hypoxic neuroprotective effect with felbamate within a wide concentration range (380 to 1,700 μM) in the hippocampal slice hypoxic model. Felbamate's hypoxic EC50 in vitro appears to fall between its anticonvulsant ED50 for MES (46 mg/kg) and metrazol (238 mg/kg) in rats. At high concentration of felbamate, no evidence of toxicity for electrophysiological function is

seen.

As previously stated, glycine augments NMDA receptor-mediated membrane depolarization and is required for activation of NMD-gated calcium channels. Furthermore, excessive activation of this "supramolecular complex" (e.g., by excitatory amino acids) has been shown to be associated with seizure disorders, ischemic brain damage and other neuropathologies. Compounds such as felbamate that interact at the strychnine-insensitive glycine modulatory site appear to alter the affects of glycine on the NMDA-gated channels. Thus, the data presented here support a mechanism for neuroprotective effects of felbamate via glycine receptor interaction.

It should be understood that the above Examples are illustrative of the best mode only of the invention herein disclosed. Given the present disclosure, it is anticipated that numerous variations will occur to those skilled in the art. A latitude of modification, substitution and change is intended and in some instances, some features of the invention will be employed without a corresponding use of other features. Accordingly, it is intended that the spirit and scope of the invention disclosed herein should be limited only by the following claims.

Claims

1. The use of 2-phenyl-1,3-propanediol for the manufacture of a medicament for treating and controlling the symptoms of, or for the prevention and control of neurodegenerative disorders associated with or resulting from excessive activation of the N-methyl-D-aspartate receptor complex in human or other warm blooded animal patients.

2. A use according to claim 1, wherein the medicament is manufactured in the form of a composition comprising also a pharmaceutical carrier, the composition being in dosage unit form.

3. A use according to claim 2, wherein the medicament is manufactured in the form of a gelatin capsule.

4. A use according to claim 2, wherein the medicament is manufactured in the form of a tablet.

5. A use according to any of claims 1 to 4, wherein the neurodegenerative disorder is Alzheimer's disease.

6. A use according to any of claims 1 to 4, wherein the neurodegenerative disorder is Parkinson's disease.

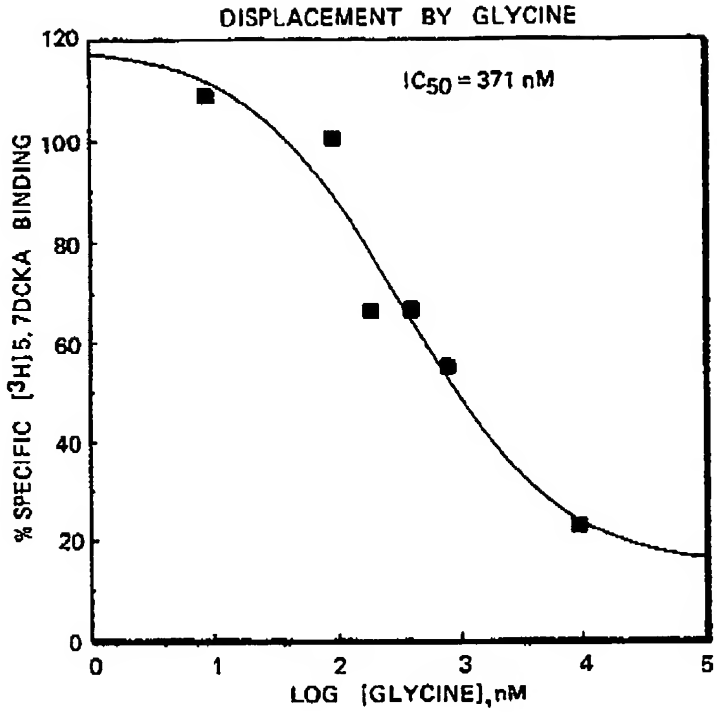
DISPLACEMENT BY GLYCINE
IC_{50} = 371 nM
% SPECIFIC [3H]5, 7DCKA BINDING
LOG [GLYCINE],nM
0
20
40
60
80
100
120
0
1
2
3
4
5

FIG. 1

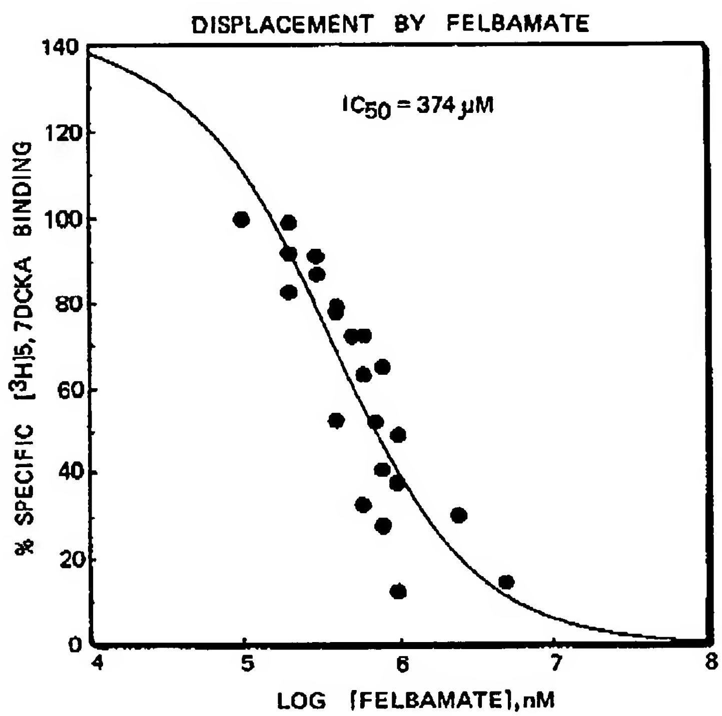
DISPLACEMENT BY FELBAMATE
IC_{50} = 374 µM
% SPECIFIC [3H]5, 7DCKA BINDING
LOG [FELBAMATE],nM
0
20
40
60
80
100
120
140
4
5
6
7
8

FIG. 2

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 92 30 7952

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
D,X D,Y	US-A-4 978 680 (ROBERT D. SOFIA) 18 December 1990 *col. 1, paragraphs 1 and 2 * * examples 1,2 *	1-4 5,6	A61K31/27
P,X	EPILEPSIA vol. 33, no. 3, 1992, pages 564 - 572; H. STEVE WHITE ET AL: 'A neuropharmacological evaluation of Felbamate as a novel anticonvulsant' * abstract *	1,2	
X	EPILEPSIA vol. 27, no. 1, 1986, pages 27 - 34; EWART A. SWINYARD: 'Comparative anticonvulsant activity and neurotoxicity of felbamate and four prototype antiepileptic drugs in mice and rats' * abstract *	1,2	TECHNICAL FIELDS SEARCHED (Int. Cl.5)
X	NEUROLOGY vol. 40, no. 4/1, 1990, page 193; R.A.WALLIS ET AL.: 'Felbamate is a potent anti-hypoxic agent in the hippocampal slice' * abstract *	1	A61K
X	DRUGS OF THE FUTURE vol. 11, no. 11, 1986, pages 931 - 932; 'felbamate' * the whole document *	1	
Y	ARZNEIMITTELFORSCHUNG/DRUG RESEARCH vol. 40, no. 1, May 1990, pages 511 - 514; L.TURSKI: 'N-Methyl-D-aspartat-Rezeptorkomplex' * summary *	5,6	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	26 OCTOBER 1992	TZSCHOPPE D.A.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
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